Посветени на хуманността
МЕДИЦИНСКИ УНИВЕРСИТЕТ – ПЛОВДИВ
4002 Пловдив, бул. Васил Априлов № 15А

www.mu-plovdiv.bg

Dedicated to humanity
MEDICAL UNIVERSITY – PLOVDIV
15A, Vasil Aprilov Blvd., 4002 Plovdiv, Bulgaria

TO
ASSOC. PROF. DR. TRIFON CHERVENKOV, DM
CHAIRMAN OF THE SCIENTIFIC JURY
DETERMINED BY ORDER No. R-109-149/13.03.2025
OF THE RECTOR OF MU-VARNA "PROF. DR. PARASKEV STOYANOV"

R E V I E W

From: Prof. Dr. Vesselina Goranova-Marinova, MD
Head of the Hematology Section, Faculty of Medicine, Medical University of Plovdiv
External member of the scientific jury according to order No. R-109-149/13.03.2025
of the Rector of Medical University of Varna "Prof. Dr. Paraskev Stoyanov"

Regarding: *procedure for acquiring the scientific and educational degree "Doctor"*

Field of higher education: 7. "Healthcare and Sports"
Professional field: 7.1. "Medicine"
Doctoral Program: "Hematology and Blood Transfusion"
Author: Dr. Radi Evgeniev Lukanov, Hematology Sector, Second Department of Internal Medicine, Faculty of Medicine, Medical University - Varna
Form of Doctoral Studies: Independent Preparation
Topic of Ph Thesis: "Biomarkers for a Personalized Approach in the Choice of Treatment for Patients with Myeloma"
Scientific Leader: Prof. Dr. Ilina D. Micheva, MD

1. General presentation of the procedure. The presented set of materials on paper and electronic media for the procedure for acquiring the Educational and Scientific Degree "Doctor" at MU-Varna is in accordance with the Regulations on the structure and activities of MU-Varna, Art. 68 para. 1 of the Higher Education Act; Art. 7, para. 1 of the Act on the Organization and Activities of MU-Varna; Art. 5, para. 1 and Art. 6, para. 1 of the Regulations for its implementation, the Regulations for the Development of the Academic Staff of MU-Varna. All necessary documents are presented, namely the orders for enrollment and expulsion of the doctoral student, the protocols of extended departmental councils and the doctoral minimum exam, which reflect all stages of the development of the doctoral student, including the dissertation itself. A list and copies of publications related to the PhD thesis are attached to the set of materials. In total, 1 full-text publication in the journal is presented. "Hematology" - the official publication of the Bulgarian Medical Academy of Agriculture, referenced and indexed in the international database "Scopus" and 2 participations in international congresses, in which the doctoral candidate is the lead author. ***The doctoral procedure has been followed.***

2. Introduction of the doctoral student. Dr. Radi Evgeniev Lukanov is a specialist in clinical hematology and an assistant professor at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. He completed his secondary education at the Mathematical High School "Dr. Petar Beron" - Varna with a profile in mathematics and English, and in 2018 he graduated as a medical doctor from the Medical University - Varna. Between 2018 and 2023, Dr. Lukanov specialized in clinical hematology at the University Hospital "St. Marina" - Varna, where since 2023 he has held the position of a specialist in hematology. The same year he successfully passed the European Exam in Hematology - a certificate for excellent knowledge in the various modules of the specialty. In parallel with his work in the Hematology Clinic, since 2019 he has been a full-time assistant professor in hematology at the Second Department of Internal Medicine of the Medical University - Varna. Since 2020 is a doctoral student in independent training at the same university with a PhD Thesis on the topic: "Role of biomarkers for the personalized approach in the diagnosis and treatment of multiple myeloma". Dr. Lukanov's research activity is focused on the field of multiple myeloma. He has participated in national and international scientific forums, including the congresses of the European Hematology Association (EHA) and the Lymphoma Leukemia and Myeloma Congress (New York, 2024), where he presented results of his research on the role of miRNA as biomarkers. Dr. Lukanov was awarded the First Prize in the "Rarities" category at the Eleventh National Congress of Hematology (2019) for a poster on POEMS syndrome. His participation in a number of student circles and scientific initiatives during his studies testifies to an early and sustained interest in scientific and academic career. An interesting fact from his biography is that Dr. Lukanov is an excellent chess player, awarded the Rector's Award of MU-Varna for his chess skills. He is a member of the Bulgarian Medical Association of Hematology and the European Hematology Association. He speaks English at level C1 and has a working level of Russian, Italian and German**. *The doctoral student has theoretical knowledge, practical experience, additional qualifications and focused scientific and practical interests in the field of doctoral studies.***

3. Relevance of the topic. The topic of the dissertation is relevant and significant in the context of modern hematological science and clinical practice. Myeloma is a hematological malignancy with a heterogeneous clinical course, therapeutic response and survival. In recent years, the efforts of the scientific community have been directed towards the introduction of personalized treatment strategies based on molecular and biological markers. The topic of Dr. Radi Lukanov's dissertation is extremely relevant, fitting into the modern paradigm of personalized medicine. The research concerns the identification and application of biomarkers that support individualized therapeutic decision-making in patients with myeloma - a problem of high clinical significance. The dissertation presents a comprehensive and systematic study

of the possibility of using specific microribonucleic acids (miRNA) as biomarkers in clinical practice for diagnosis, monitoring of therapeutic response and prediction of outcomes in patients with multiple myeloma. *The topic has not been developed on a national level. The topic of the dissertation is important, topical, and of great clinical significance.*

4. Knowledge of the scientific problem. The presented scientific hypothesis suggests that new approaches for a more precise understanding of the biological processes in multiple myeloma are gaining increasing importance. Among the most important areas are studies related to the role of miR as key regulators of gene expression, as well as various epigenetic factors that influence the growth of malignant cells. Of particular importance is the interaction between myeloma cells and the tumor microenvironment in the bone marrow, including the role of miRNA in the processes of immunomodulation and angiogenesis. The author develops the scientific hypothesis that selected miRNAs can be identified as new biomarkers for early diagnosis, more accurate prognosis and optimal monitoring of therapeutic response, with the potential to lead to more precise risk stratification and improved clinical outcomes in patients with multiple myeloma*. The author knows the scientific problem in depth, can formulate a scientific hypothesis and conduct an analysis, the results of which would lead to improved diagnosis and monitoring of patients with multiple myeloma as a prerequisite for adapting the therapeutic choice and extending their survival.*

5. **Methodology of the study.** The scientific study is conducted as a single-center and prospective study at the Hematology Clinic, "St. Marina" University Hospital - Varna for the period 2022 - 2024 on 56 patients and 12 healthy controls. The methodology applied is modern, adequate to the objectives of the study and includes valid clinical, laboratory and statistical methods. The baseline data and demographics, the criteria for inclusion and exclusion of patients are described in detail. The approach has a clearly expressed applied focus and potential for application in clinical practice. The analyzed 56 patients with multiple myeloma have undergone therapy according to a standard protocol. The analysis of miRNA in patients with multiple myeloma using the methods of RNA isolation and RT-PCR is an innovative approach that has not been conducted in the country so far. The possibility of collecting samples and examining miRNA in patients who have achieved different depths of therapeutic response offers a good basis for assessing the diagnostic and prognostic potential of the analyzed biomarkers**. The laboratory methods used, as well as the statistical analyses, are appropriate for the purposes of the study, allowing for obtaining reliable results.**

6.Characteristics and evaluation of the dissertation work. The dissertation work is developed on 134 standard pages. It contains 15 tables and 34 figures. 214 literary sources are cited, selected purposefully. Not a single publication by Bulgarian authors is indicated,

although national author teams develop various problems of myeloma disease and its complications in a scientific aspect. The mandatory sections of the scientific work are professionally developed, maintaining an acceptable ratio between them. Clear and precise, grammatically correct Bulgarian language is used.

6.1 Literature review. The literature review is presented on 33 standard pages, in-depth and focused on the scientific problem. It sets the context of the study of mi RNA as biomarkers, starting with a brief presentation of multiple myeloma (MM) and the importance of finding new biomarkers for the diagnosis and prognosis of the disease. It is emphasized that despite advances in therapy, multiple myeloma remains a challenge for treatment, which justifies the need for new diagnostic tools. The inclusion of miRNAs as potential biomarkers for early detection and prediction of response to treatment is fully justified, emphasizing the importance of this research direction. The literature data on the importance of miR-126-5p and miR-199a-5p are supported by data from several studies. The logical transition from the general description of the disease to specific molecules is well made, as Dr. Lukanov presents the mechanism by which miRNAs act as tumor suppressors. The dissertation provides evidence for a decrease in miRNA levels in patients with newly diagnosed MM compared to healthy controls. The references to the existing literature linking these miRNAs to various types of neoplastic disease are well-founded and support the hypothesis that their expression may be key in the pathogenesis of MM. The positioning of miRNA-126 and miRNA-199a as tumor suppressors with mechanisms for inhibiting angiogenesis and cell proliferation is logical and supported by citations from existing studies on the topic. Thus, Dr. Lukanov presents the role of miRNA and their part in the molecular mechanisms of MM, which contributes to the comprehensive understanding of their potential in clinical practice. **The literature review concludes with a motivated justification for the need for additional studies to clarify the role of miRNA in multiple myeloma and the possibility of their use in clinical practice for monitoring the evolution of the disease and the effect of the treatment.**

6.2 Aim and tasks. The formulated main goal is clearly defined and adequately supported by 14 specific tasks, which are consistently solved in the course of the study. They reflect the well-chosen scientific issues and structure the logic of the study. The goal of the dissertation is clearly formulated and justified – to study the role of selected biomarkers for optimizing the therapeutic choice in patients with multiple myeloma. The tasks are specified logically and reflect the sequence of the research process. They cover both clinical and laboratory aspects, including analysis of prognostic factors and assessment of therapeutic response.

6.3 Section "Material and Methods". The section "Material and Methods" is presented on 8 standard pages. Patients with MM (56) and the control group (12) were examined in two time

periods - at diagnosis and at month 6 after standard treatment for the myeloma group. Patients were examined with routine methods for the diagnosis of multiple myeloma. Specific analyses of selected miRNAs were performed with RNA isolation and Real-time PCR, performed using the miRNeasy Serum/plasma Kit (Qiagen) reagent. The study was conducted in accordance with the manufacturer's protocol, which includes 2 stages - RNA isolation and real-time polymerase chain reaction. Statistical methods were correctly selected and allowed obtaining reliable results. Descriptive, variative, comparative, correlation, regression, ROC analyses were used, as well as the Shapiro-Wilks tests for assessing the normal distribution and χ 2 nonparametric test for agreement. The Kaplan-Maier method with log-rank test was applied to analyze the time to disease progression or lethal outcome. The data were processed and visually presented using GraphPad Prism, version 8.0.2 for Windows, USA and IBM and SPSS Statistics v. 23. *I fully and without comments accept the section "Material and Methods"*

6.4. The section "Results" is presented on 33 standard pages. The presentation of the section is presented in a clear manner in tables, figures, graphs and diagrams. The sequence of the tasks set is logically followed. Demographic data and clinical characteristics of patients with MM are presented. The results are clearly presented, statistically processed and interpreted in the context of modern scientific literature. The author demonstrates an ability for critical analysis and presents both the positive findings obtained and the limitations of the study. From the presented data, I note the following results as having particularly important clinical significance:

1. The correlation between miR-126-5p and beta-2 microglobulin levels can provide valuable additional information for determining the prognosis and monitoring the evolution of the disease. The weak correlation between miRNA and serum creatinine is in the same context, without independent significance.

2. miR-126-5p shows a significant increase after treatment in patients who achieved a complete and very good partial therapeutic response. On the one hand, this can be an element of the assessment of the efficacy of the therapy, and on the other hand, in a pathogenetic aspect, it has the potential to disrupt the proangiogenic environment and slow the progression of the disease. Unlike miR-126-5p, miR-199a-5p does not show significant changes after treatment, which suggests that although useful for making the diagnosis, it does not represent a dynamic marker for the short-term therapeutic response.

3. The use of ROC analysis and AUC evaluation demonstrated that miR-126-5p and miR-199a-5p have high sensitivity and specificity and offer potential for use in clinical practice. On the other hand, miR-214-3p and miR-497-5p show low diagnostic value, which is an important result for the proper planning of future studies.

4. The assessment of OS and PFS against the levels of miR-214-3p and miR-497-5p as predictive markers for disease progression and survival has clinical value, especially as risk factors for early mortality.

I accept the results obtained without critics. As results of important clinical significance, I note the data that of all the biomarkers studied, the most sensitive and significant for response to treatment is miR-126-5p, while miR-214-3p and miR-497-5p are unfavorable prognostic markers.

6.5 Section "Discussion". In 11 standard pages, the doctoral student analyzes his own results, discusses their significance and compares them with those of other author teams. The discussion is well written, with clear connections between the scientific conclusions and the possibilities (or lack thereof) for practical application, which is a strong point of this scientific work. The author discusses important aspects of miRNA dynamics during treatment and prediction of outcomes in patients with multiple myeloma (MM). An example of this is the increase in miR-126-5p levels in patients with complete response or very good partial response in support of the hypothesis that effective treatment can restore miR-126-5p expression, which has the potential to disrupt the proangiogenic environment and slow disease progression. Unlike miR-126-5p, miR-199a-5p does not show significant changes after treatment and is not relevant as a dynamic marker for short-term therapeutic response. The author discusses miR-214-3p and miR-497-5p as negative prognostic biomarkers that can be used to predict poor outcomes in MM and shorter survival. He points to the literature data that establish that miR-214-3p may play a role in disease progression by activating the PI3K/AKT pathway, which increases Tu cell survival and resistance to apoptosis, and miR-497-5p, although known as a tumor suppressor factor in some diseases, may have a dual effect in MM by inducing pathological angiogenesis or impairing apoptosis of myeloma cells. The author explains the lack of significant results in the analysis of miR-373-3p with technical limitations, its absence in significant amounts in the samples, or minimal involvement of miR-373-3p in the pathophysiology of the disease. The author points out the potential of miRNA in the monitoring of the treatment of MM and discusses future research and perspectives in the context of possible therapeutic strategies through miRNA modulation, such as restoring the levels of miR-126-5p and miR-199a-5p or inhibiting oncogenic miRs. *I accept the "Discussion" section without remarks*

6.6. The conclusions are 6 in number and clearly follow the set goals and objectives. The conclusions emphasize the importance of microRNA as biomarkers, which can help not only diagnose and monitor the disease, but also improve the individualization of the therapeutic approach in patients with MM. *I agree with the conclusions formulated in this way.*

6.7. Bibliography. 214 literary sources are cited, selected purposefully, but not a single publication by Bulgarian authors is indicated. The analyzed scientific publications in the last 5 years are 20% of the total number. These data testify to the relevance of the problem and the great research interest in the topic**.**

7. Assessment of the contributions of the dissertation work. The dissertation work ends with the presentation of contributions that have a scientific, as well as scientific-applied nature. five of the contributions are of original nature and 4 of applied scientific value**. *I accept the presented contributions.***

8. Personal participation of the doctoral student. The doctoral student has personal participation in the formulation of the scientific idea, the collection of the material and the design of the study. His personal participation is in the statistical processing of the data and the analysis of the obtained results. The conclusions and contributions are also drawn with the participation of Dr. Lukanov. The doctoral student is the first author in scientific communications related to the dissertation. ***The doctoral student has a major personal participation in the development of the dissertation.***

9. Autoreferat. The autoreferate contains 71 pages, provides an overall picture of the dissertation work, fully reflecting the individual sections. All figures and tables are included, except those from the literature review, and present the necessary data in sufficient volume.

10. Critical notes and recommendations:

1. Although the dissertation examines the significance of miR-126-5p and miR-199a-5p as biomarkers, additional studies are needed in larger patient populations to confirm the results and clarify the mechanism of their action in multiple myeloma.

2. Additional studies are needed to substantiate the practical applicability of miR-214-3p and miR-497-5p in clinical settings, especially in the stratification of patients by risk groups.

3. The dissertation candidate should increase his publication activity on the topic

4. No literary sources on the topic by Bulgarian authors are cited

11. Conclusion. The dissertation work of Dr. Radi Evgeniev Lukanov meets the requirements of the ZRASRB, the Regulations for its implementation and the Regulations of MU-Varna for awarding the scientific and educational degree "Doctor". The dissertation work represents a solid and innovative study of the role of miRNA as biomarkers in multiple myeloma. The scientific work shows potential for further development of prognostic models and therapeutic strategies based on molecular markers, and may have a significant impact on the future diagnosis and monitoring of the disease in case it finds confirmation in larger cohorts of patients with MM. The results provided are adequate and valuable for the scientific community and clinical practice. The dissertation work of Dr. Radi Lukanov demonstrates established qualities for analysis and synthesis of scientific information, the ability to

formulate conclusions and build scientific hypotheses. In-depth scientific knowledge and practical skills in the specialty "Hematology and Blood Transfusion" are evident. I give my positive assessment of the dissertation work on the topic "Biomarkers for a personalized approach in the selection of treatment for patients with myeloma disease" and I propose to the esteemed scientific jury to vote "for" awarding the scientific and educational degree "Doctor" to Dr. Radi Evgeniev Lukanov.

PROF. DR. VESELINA GORANOVA-MARINOVA, DM
/Reviewer /